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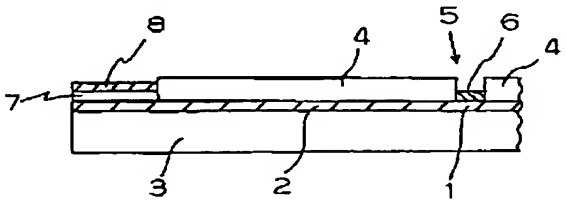
(54)【発明の名称】　一体化複合電極

(57)【要約】

【目的】　神経細胞の多点同時刺激・記録を長期にわたり行うことができ、応答性の優れた一体化複合電極を提供する。

【構成】　硬質ガラスの絶縁基盤３上の全面にＩＴＯ膜を蒸着し、８×８の格子上の各交点に各電極１の中心部が位置し、各電極の最近接の電極の中心間距離が等しく、しかもリード線２が放射状に伸びた形状にＩＴＯ膜をエッチングする。ついで、絶縁層４としてネガティブフォトセンシティブポリイミドをスピンコートし、各電極の中心に一辺５０μｍの正方形の孔５ができるように、絶縁層パターンを露光形成する。さらに、各電極の露出部分（すなわち１辺５０μｍの正方形の内部）に、膜厚５００オングストロームとなるように金６を蒸着する。リード線２の電極１と反対方向の端部近傍の部分の外部回路との接点は、金７およびニッケル８でコートする。



1

【特許請求の範囲】

【請求項1】　絶縁基盤上に、最近接の電極間距離が相等しい複数個の電極を備え、前記電極からリード線を略放射状に配設した配線部と、前記リード線をカバーする絶縁層とを設け、かつ電極面積が3×10^{2} μm^{2} 以上4×10^{4} μm^{2} 以下の範囲であり、電極部の表面抵抗が10Ω／cm^{2} 以下である一体化複合電極。

【請求項2】　最近接の電極間距離が、10μm以上10000μm以下の範囲である請求項1に記載の一体化複合電極。

【請求項3】　リード線をカバーする絶縁層が、各電極上に孔を有し、かつリード線の外部回路との接点近傍を除いて前記絶縁基盤のほぼ全面に設けられた絶縁層である請求項1または2に記載の一体化複合電極。

【請求項4】　複数個の電極中心部が、8×8の格子上の各交点に位置する請求項1～3のいずれかに記載の一体化複合電極。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、生体活動の電気的計測、特に神経細胞の電気的活動を計測する神経生理の分野で用いる、多電極を有する一体化複合電極に関する。

【0002】

【従来の技術】近年、神経細胞の医学的検討や電気素子としての適用の可能性の検討などが活発に行われてきている。神経細胞が活動する際には、活動電位が発生する。活動電位は、神経細胞のイオン透過性の変化に伴い、細胞膜内外のイオン濃度が変わることにより生じるものである。そして電極により、神経細胞近傍のイオン濃度変化（すなわちイオン電流）に伴う電位変化を測定することによって、神経活動の検出、検討が行われている。

【0003】従来、神経細胞の電気的活動を計測するには、ガラス電極等からなる記録電極と、金属電極等からなる刺激電極とを各々細胞内または細胞間に挿入し、刺激電極より刺激電流（または電圧）を印加した際の、神経細胞の電気的活動を記録電極により計測するのが普通であった。

【0004】これ以外にも、例えば細胞体を細管状のガラス吸引電極で突き刺し、細胞体の内部をガラス吸引電極中の液で還流し、このガラス吸引電極から電気信号を与えて細胞膜の電気的特性を観察するいわゆるパッチクランプ法等多数の変法がある。

【0005】さらには、絶縁性の基盤上にITO（酸化インジウム錫）等の導電性物質で直径15～20μmの電極を形成し、この上で神経細胞を培養することにより、細胞に電極を刺入する事なく、細胞に電気的刺激を印加し、また神経細胞の電気的活動を記録する方法についても本発明者らが別途提案している。

【0006】また、この改良法として、電極の直径を20～200μmとすれば、神経細胞に定電流刺激を印加した際に電極間に発生する電位差が小さくなり、この結果ITOの破壊が起こりにくく、より長期にわたる観察が可能となることも本発明者らが別途提案している。

2

【0007】

【発明が解決しようとする課題】上述した従来の技術およびその変法においては、ガラス電極など、細胞に比べてかなりの大きさにならざるを得ない電極を用いるので、おもに空間的な制約と操作精度上の制約で、1つのサンプル中に一度に2本以上の記録電極を挿入し、神経細胞の電気的活動を記録する多点同時計測は非常に困難であるという課題があった。

【0008】神経回路網全体の働きを検討するためには、多くの神経細胞の活動を同時に記録する必要があり、測定点が増えるにしたがって、困難さの度合が増加し、多細胞間の観察ができ難いという課題があった。

【0009】さらには、ガラス・金属等の電極を細胞内または細胞間に刺入する必要があるために、細胞に与える損傷が大きく、数時間以上の長時間にわたる測定ができ難いという課題があった。

【0010】一方、絶縁性の基盤上にITO等の導電性物質で直径（または1辺）15～20μmの円形（または正方形）の電極を形成したものを用いれば、多細胞間にわたる信号伝達の観察が可能となる。しかしながら、電極面積が177μm^{2} ～400μm^{2} と小さいため、培養液界面での電極抵抗は数MΩとなり、通常刺激は定電流で与えられるので、電気抵抗が大きいと電極間にはきわめて大きな電位差が発生することになり、かかる大きな電圧で長期にわたり電気刺激を与えるとITOの破壊がおき、このため長期にわたる観察が困難であるという問題点があった。

【0011】また、電極面積を300μm^{2} ～40000μm^{2} にすれば、培養液界面での電極抵抗が小さくなるため、電極間に発生する電位差は比較的小さなものとなる。長期にわたり刺激電流を加えてもITOの破壊は、顕微鏡的には認められなかった。しかしながら、ある電極から刺激電流を印加し、他の電極で刺激に伴う電位変化を記録した際、長期刺激の前後で記録波形に大きな変化がみられた。すなわち長期刺激後では、刺激電流印加が記録波形に及ぼす影響（すなわちアーチファクト）が、長期刺激前より大きくなった。波形変化の原因は、電極表面が分極することによると考えられる。最悪の場合、神経細胞の電気的活動はアーチファクトに隠れ測定不可能となった。また、アーチファクトがそれほど大きくならない場合でも、長期刺激前後で神経活動強度を比較することが困難となるという問題点があった。

【0012】本発明は、かかる従来の問題点を解決し、神経細胞などの多点同時刺激・計測を簡便に行い、多細胞間にわたる信号伝達観察を数時間以上にわたり可能とし、かつ刺激電流印加に伴うアーチファクトの発生を抑

3

え、長期刺激の前後にわたり電位記録波形の比較を可能ならしめる一体化複合電極を提供することを目的とする。

【0013】

【課題を解決するための手段】上記課題を解決するため、本発明の一体化複合電極は、絶縁基盤上に、最近接の電極間距離が相等しい複数個の電極を備え、前記電極からリード線を略放射状に配設した配線部と、前記リード線をカバーする絶縁層とを設け、かつ電極面積が3×10^2 μm^2 以上4×10^4 μm^2 以下の範囲であり、電極部の表面抵抗が$10\Omega/cm^2$ 以下である構成を有する。

【0014】前記本発明の一体化複合電極においては、最近接の電極間距離が、10μm以上1000μm以下であることが好ましい。また、前記本発明の一体化複合電極においては、リード線をカバーする絶縁層が、各電極上に孔を有し、かつリード線の外部回路との接点近傍を除いて前記絶縁基盤のほぼ全面に設けられた絶縁層であることが好ましい。

【0015】また、前記本発明の一体化複合電極においては、複数個の電極中心部が、8×8の格子上の各交点に位置することが好ましい。

【0016】

【作用】本発明の一体化複合電極は、絶縁基盤上に、最近接の電極間距離が相等しい複数個の電極を備え、前記電極からリード線を略放射状に配設した配線部と、前記リード線をカバーする絶縁層とを設け、かつ電極面積が3×10^2 μm^2 以上4×10^4 μm^2 以下の範囲であり、電極部の表面抵抗が$10\Omega/cm^2$ 以下であるので、本発明の一体化複合電極上に培養した神経細胞に信号を与え、同時に細胞間の信号の伝達を計測する際に、最近接の電極間距離を測定対象の神経細胞（すなわち細胞体と樹状突起と軸索突起）の長さとほぼ等しく調整し、しかもこの電極を等間隔で並ばせることにより、一細胞体が電極上に配置し、この細胞体から伸びた細胞突起を介した細胞体が、隣合う電極上に位置する確率が高くなる。したがって、隣合う細胞体間の信号の伝達を検知できる。

【0017】しかも、電極から伸ばしたリード線を略放射状に配置したので、例えばリード線を平行に配置した場合に比べて、リード線間の容量成分（キャパシタンス）が少なくなり、電気信号であるパルス信号波形の崩れを小さくでき、回路の時定数が小さくなるため、早いパルス信号に対する応答性が向上し、神経細胞活動の早い成分に対する追従性が向上する。

【0018】さらに、電極面積を3×10^2 μm^2 以上4×10^4 μm^2 以下の範囲で調整することにより、数時間以上の長時間にわたり細胞に電気刺激を与え、かつ細胞の電気的活動を測定することができる。

【0019】また、電極部の表面抵抗が$10\Omega/cm^2$

4

以下であるため、ある電極で神経細胞に長期に刺激電流を印加し、他の電極で刺激電流に応じた神経細胞の電気的活動（電位変化）を記録する際に、刺激電極表面の分極が起こり難いため、刺激電流が電位記録波形に及ぼす影響（すなわちアーチファクト）が小さくなる。特に、長期に刺激電流を印加した後でもアーチファクトが小さく、かつ形態の変化が無いため、長期刺激前後での神経細胞の電気的活動を比較することができる。

【0020】また、前記本発明の一体化複合電極において、最近接の電極間距離が、10μm以上1000μm以下である好ましい態様とすることにより、一般的に神経細胞の神経突起の長さがこの範囲内であるので、細胞体が電極上に位置し、かつ神経突起を介して結合する可能性が高く、神経細胞の測定に好都合な電極間距離となる。

【0021】また、前記本発明の一体化複合電極において、リード線をカバーする絶縁層が、各電極上に孔を有し、かつリード線の外部回路との接点部近傍を除いて前記絶縁層基盤のほぼ全面に設けられた絶縁層である好ましい態様とすることにより、絶縁層をリード線上のみに選択的に設ける場合に比べ、感光性樹脂からなる絶縁性材料を使用して、ほぼ全面にこの樹脂を塗布し、フォトエッチング手法により、各電極上の絶縁層を除去して電極が露出するように孔を開けるなどのフォトエッチングで容易に必要な絶縁層が形成でき、生産を容易にすることができるし、絶縁不良の確率を小さくできるので好ましい。

【0022】さらにまた、前記本発明の一体化複合電極においては、複数個の電極中心部が、8×8の格子上の各交点に位置することにより、前記本発明の電極からリード線を略放射状に配設できる最高の電極数とすることができるので好ましい。

【0023】

【実施例】本発明に供される絶縁基盤材料としては、細胞培養後顕微鏡観察する必要があるため透明な基盤が好ましく、石英ガラス、鉛ガラス、ホウ珪酸ガラス等のガラス、もしくは石英等の無機物質、または、ポリメタクリル酸メチルまたはその共重合体、ポリスチレン、ポリ塩化ビニル、ポリエステル、ポリプロピレン、尿素樹脂、メラミン樹脂などの透明性を有する有機物質等が挙げられるが、機械的強度と透明性を加味すると無機物質が好ましい。

【0024】本発明に供される電極材料としては、例えば酸化インジウム錫（ITO）、酸化錫、Cr、Au、Cu、Ni、Al等が使用可能である。特に、ITOもしくは酸化錫を用いると、電極は僅かに黄味を帯びた透明なものとなり、神経細胞の顕微鏡下での視認性がよく、実験操作上有利であるが、とりわけITOが良導伝性であるため望ましい。

【0025】リード線材料にも同様の材料が適用でき、

5

やはり電極材料と同様の理由でＩＴＯが好ましい。特に限定するものではないが、通常これらの電極やリード線の厚みは、約５００～５０００オングストローム程度であり、通常これらの材料を絶縁基盤上に蒸着し、フォトレジストを用いてエッチングにより所望のパターンに形成できる。

【００２６】また、本発明に供されるリード線を絶縁するための絶縁層材料としては、例えばポリイミド（ＰＩ）樹脂、エポキシ樹脂、アクリレート樹脂、ポリエステル樹脂、またはポリアミド樹脂等の透明な樹脂が挙げられる。

【００２７】これらの樹脂は、リード線上に通常の手法によって塗布して絶縁層が構成される。なお、絶縁層材料が光重合性等の感光性樹脂であると、前述したように電極を露出させるために電極上の絶縁層部分に孔を開けるなどのパターン形成が可能となるため好ましい。

【００２８】特に、絶縁層材料がＰＩであり、培養する細胞が神経細胞である場合には、良好な生育を示すため望ましい。さらにＰＩの中でも、ネガティブフォトセンシティブポリイミド（ＮＰＩ）が、配線部のパターン形成と同様に、略全面にネガティブフォトセンシティブポリイミドを塗布した後フォトエッチングプロセスを用いて電極状に孔を形成できるため好ましい。

【００２９】また、絶縁層の厚みは絶縁性が付与できる程度であればよく、特に限定するものではないが、通常０．１～１０μmが好ましく、１～５μmがさらに好ましい。

【００３０】本発明の一体化複合電極を用いて、直接細胞を培養して細胞の電気活動を計測記録した。培養条件もしくは細胞の種類によって、細胞体の大きさもしくは樹状突起や軸索などの細胞突起の長さが異なるが、一体化複合電極の最近接の電極間距離は、１０～１０００μmが好ましい。電極間距離が１０μm未満であると、互いに近接し過ぎるため細胞体が細胞突起を介して相隣合う確率が減り、またリード線の配線も困難となる。また、１０００μmを越えると、リード線の配線はしやすいが、細胞突起が１０００μm程度も伸びることは稀なため、細胞体が電極上に位置する確率が減る。一般の条件でも、培養した細胞の細胞突起の長さは、哺乳動物の中枢神経細胞の場合、平均２００～３００μm程度であるため、電極間距離は２００～３００μm程度が望ましい。

【００３１】電極面積については、長期にわたり細胞に電気刺激を印加する際の電極破壊を避けるため、培養液との界面での抵抗を小さくする必要があるため、ある程度以上の大きさが要求される。しかしながら、電極面積が大きくなり培養液との界面での抵抗が小さくなると、測定される細胞の電気的活動は小さくなり、Ｓ／Ｎ比が低下する。すなわち、電流値Ｉが一定とすると、Ｉ＝Ｖ／Ｒであるから、抵抗値Ｒが小さくなると測定される電

6

位Ｖの変化も小さくなる。つまり測定される細胞の電気的活動が小さくなりＳ／Ｎ比が低下する。このため、電極面積は慎重に調整される必要があり、円形状の電極の場合、直径が２０μmより大きく２００μm以下、特に１００μm～２００μmが好ましい。

【００３２】また、電極部分の表面抵抗を１０Ω／cm^2以下にするため、ＩＴＯ上面に金属をコートした。コート材料としては、Ａｇ，Ａｌ，Ｂｉ，Ａｕ，Ｃｕ，Ｃｒ，Ｐｔ，Ｃｏ等が使用可能であるが、神経細胞に対する毒性の低さを考慮すれば、Ａｕ，Ｐｔの使用が望ましい。コートの厚みは、特に限定されるものではないが、約５００オングストローム程度であり、通常これらの材料を絶縁基盤上に蒸着し、フォトレジストを用いてエッチングにより所望のパターンに形成できる。

【００３３】さらに、本発明の前述した好ましい態様によれば、一体化複合電極の絶縁層中の孔は、一体化複合電極上で培養した細胞体に電気刺激を与えると同時に、隣合う細胞体から電気的活動を検知するため、電極を露出する目的で形成し、電極中心部に位置する。

【００３４】また、電極から伸ばしたリード線を略放射状に配設することにより、リード線間の容量成分がなくなり、ノイズが減少し測定精度が向上する。また、本発明の一体化複合電極の電極中心部が、同心円状もしくは８×８以下の格子上の各交点に位置する構成であると、リード線を放射状に配線でき、特に可能な限り多くの電極を構成し、多点同時刺激・記録を行うという観点からは、８×８の格子上の各交点に電極を設けることが望ましい。

【００３５】以下具体的実施例で、本発明の一体化複合電極をさらに詳細に説明する。

実施例１

図１は絶縁基盤３上に電極１とリード線２を形成した本実施例の一体化複合電極の絶縁層のない状態の配線部のパターンを示した平面図である。図２は図１で示した部材の上に形成された絶縁層のみの平面図の一部切り欠き図である。図３は本実施例の一体化複合電極の一部の断面図である。以下これらの図面を参照しながら説明する。

【００３６】まず、複合電極配線部の作製について述べる。一体化複合電極の絶縁基盤３は機械的強度の強い透明な絶縁素材として、５０×５０×１mmの硬質ガラス（“ＩＷＡＫＩ　ＣＯＤＥ　７７４０　ＧＬＡＳＳ”［岩城硝子（株）製］以下同じ）を用いた。

【００３７】電極１およびリード線２の材料にＩＴＯを用い、前記硬質ガラスの絶縁基盤３上の全面に約１０００オングストローム厚に蒸着し、その後洗浄した。次に、８×８の格子上の各交点（図２の５で示されたような位置）に各電極１の中心部が位置し、各電極の最近接の電極の中心間距離が等しく、しかもリード線２が放射状に伸びた形状の電極１およびリード線２のパターンに

7

なるように、フォトレジストを用いて露光し、純水５０、塩酸５０、硝酸１の体積比で混合した溶液中でＩＴＯをエッチングした後、フォトレジストを除去した。電極１の直径は６０μm、リード線２の幅は３０μm、電極中心間距離は３００μmの配線部を形成した。

【００３８】ついで、絶縁層４としてネガティブフォトセンシティブポリイミド（以下ＮＰＩと略す）を、乾燥後の厚みが１μmとなるようにスピンコートし、図２に示すように配線部の各電極の中心に１辺５０μmの正方形の孔５ができるように、絶縁層パターンを露光形成した。さらに、各電極の露出部分（すなわち１辺５０μmの正方形の内部）に、膜厚５００オングストロームとなるように金６を蒸着した。

【００３９】リード線２の電極１と反対方向の端部近傍の部分の外部回路との接点は、金７およびニッケル８でコートし、耐久性を向上させた。なお、本実施例では電極１およびリード２の部分にＩＴＯ、絶縁層にＮＰＩ、電極表面コート材に金を用いたが、用いる材料はこれらに限定されないことは既に述べた。

【００４０】また、本発明の一体化複合電極を構成するためのプロセスは本実施例の方法に限定されない。

実施例２

次に、一体化複合電極上での神経細胞の培養について述べる。

【００４１】実施例１のようにして構成した一体化複合電極上で、神経細胞としてラット大脳視覚皮質を培養した。以下、培養法について詳細に述べる。

（イ）妊娠後１６～１８日を経過したＳＤラットの胎児の脳を摘出し、氷冷したハンクス平衡塩液（以下ＨＢＢＳと略す）に浸す。

（ロ）氷冷ＨＢＢＳ中の脳から視覚皮質を切り出し、イーグル最小必須培地（以下ＭＥＭと略す）液中に移す。

（ハ）ＭＥＭ液中で、視覚皮質をできるだけ細かく、最大でも０．２mm角となるように切断する。

（ニ）細かく切断した視覚皮質を遠沈管（遠心分離用試験管）に入れ、カルシウムおよびマグネシウムを含まないＨＢＢＳ（以下ＣＭＦ－ＨＢＢＳと略す）で３回洗浄した後、適量の同液中に分散する。

（ホ）上記（ニ）の遠沈管中に、トリプシンのＣＭＦ－ＨＢＢＳ溶液（０．２５重量%）を加え、全量を倍にする。緩やかに撹拌しながら、３７℃で１５分から２０分間恒温状態に保ち酵素反応を行わせた。

（ヘ）牛胎児血清（ＦＣＳ）１０vol.%を含むダルベッコ変更イーグル培地（ＤＭＥＭ）とＨａｍＦ－１２培地を１対１の体積比で混合したＤＭＥＭ／Ｆ－１２混合培地を、上記（ホ）を経た遠沈管中に加え、全量をさらに倍にする。先端をバーナーであぶり口径を小さくしたパスツールピペットで、緩やかにピペッティングを繰り返し（最大２０回程度）、細胞をほぐす。

（ト）９８０６．６５m／ｓｅｃ^2（すなわち１０００

8

g）で約５分間遠心分離を行う。遠心分離終了後、上清を捨て、沈澱をＦＣＳ５vol.%を含むＤＭＥＭ／Ｆ－１２混合培地に懸濁する。

（チ）上記（ト）および（チ）をあと２回（計３回）繰り返す。

（リ）最終的に得られた沈澱を、５vol.%ＦＣＳを含むＤＭＥＭ／Ｆ－１２混合培地に懸濁し、懸濁液中の細胞濃度を赤血球計数板を用いて計測する。同様の培地を用いて細胞濃度を２～４×１０^6 個／ｍｌになるように調整する。

（ヌ）一体化複合電極上に直径２５mm、高さ６mmのプラスティック製円筒を、複合電極の中心とプラスティック円筒の中心を合わせて接着することにより構成した細胞培養用ウェル中に、あらかじめ５vol.%ＦＣＳを含むＤＭＥＭ／Ｆ－１２混合培地５００μｌを加え、CO_2 インキュベータ内（空気濃度９５vol.%、CO_2 濃度５vol.%、相対湿度９７%、温度３７℃）で暖めておく。

（ル）上記（ヌ）のウェル中に、細胞濃度を調整した懸濁液１００μｌを静かに加え、再びCO_2 インキュベータ内に静置する。

（ヲ）上記（ル）の操作より３日後に、培地の半量を新しいものと交換する。交換培地はＦＣＳを含まないＤＭＥＭ／Ｆ－１２混合培地を用いる。

（ワ）以降、４～５日毎に上記と同様の培地交換をおこなう。

【００４２】これら一連の操作により、一体化複合電極上でラット大脳視覚皮質の神経細胞を培養することができた。細胞は絶縁層（ＮＰＩ）上でも白金黒を析出させた電極上でも良好に生育した。したがって、適当な位置にある電極を刺激電極または記録電極として用いれば、神経細胞電気活動の同時多点計測が可能であった。

【００４３】また、本発明の一実施例の一体化複合電極の適当な位置にある電極を通じて１００μＡの定電流刺激を１Ｈｚの頻度で１週間にわたって与えた前後で、適当な位置にある電極で神経細胞の電気的応答（電位変化）を記録した例を図４および図５に示す。図４は刺激前の神経細胞の電気的応答の記録、図５は刺激後の神経細胞の電気的応答の記録を示す。

【００４４】さらに、図６および図７に電極表面を金でコートしていない一体化複合電極を用いて、上記と同様の条件で長期刺激を加えた前後での、神経細胞の電気的応答を記録した例を示す。図６は刺激前の神経細胞の電気的応答の記録、図７は刺激後の神経細胞の電気的応答の記録を示す。

【００４５】図４から図７において、矢印は刺激電流印加に伴い発生したアーチファクト、矢頭は神経細胞の電気的活動により発生した電位変化を示す。図６から分かるように、電極表面を金でコートしていない一体化複合電極を用いた場合はアーチファクトの発生が大きいのに

9

対し、図４の本発明の一実施例の一体化複合電極を用いた場合では、アーチファクトの発生が抑えられている。

【００４６】また、図７から分かるように、電極表面を金でコートしていない一体化複合電極を用いた場合はアーチファクトの発生が刺激前より大きく、神経細胞の電気的活動はアーチファクトに隠れ測定不可能となった。それに対し、図５の本発明の一実施例の一体化複合電極を用いた場合では、図４で示された場合と同様、アーチファクトの発生が抑えられており、神経細胞の電気的活動を十分に記録することができた。

【００４７】なお、神経細胞の培養法は本実施例以外にも多くの変法があり、本実施例に限定されるものではない。

【００４８】

【発明の効果】以上説明した通り、本発明の一体化複合電極は、神経細胞の培養が可能で、従来不可能または非常に困難であった神経細胞電気活動の同時多点計測および多細胞にわたる信号伝達の数時間以上の長期観察が実現でき、また、応答性の優れた一体化複合電極を提供できる。

【００４９】また、最近接の電極間距離が、１０μｍ以上１０００μｍ以下の範囲であることにより、各細胞体が各電極上に位置し、かつ神経突起を介して結合する可能性が高くでき、神経細胞の測定に好都合な一体化複合電極とすることができる。

【００５０】また、リード線をカバーする絶縁層が、各電極上に孔を有し、かつリード線の外部回路との接点近傍を除いて前記絶縁基盤のほぼ全面に設けられた絶縁層であることにより、感光性樹脂からなる絶縁材料を使用して、ほぼ全面にこの樹脂を塗布し、フォトエッチング手法により、容易に必要な絶縁層パターンが形成でき、生産が容易で、絶縁不良の確率の小さい一体化複合電極とすることができる。

【００５１】また、各電極部分の表面抵抗が低く、かつ細胞毒性の低い物質でコートされているため、適当な電極を用いて刺激電流を加え、他の適当な電極を用いて電位変化を記録する際に、長期にわたり刺激を加えた後でも電極の分極が少なく、安定した記録が可能な一体化複

10

合電極とすることができる。

【００５２】また、複数個の電極中心部が、８×８の格子上の各交点に位置することにより、前記本発明の電極からリード線を略放射状に配設できる最高の電極数とすることができる。

【図面の簡単な説明】

【図１】本発明の一実施例の絶縁基盤上に電極とリード線を形成した本発明の一体化複合電極の絶縁層のない状態のパターンを示した平面図である。

【図２】本発明の一体化複合電極の一実施例の絶縁層のみの平面図の一部切り欠き図である。

【図３】本発明の一体化複合電極の一実施例の一部の断面図である。

【図４】本発明の一体化複合電極の一実施例において、適当な電極を用いて長期に刺激電流を印加する前に、他の適当な電極を用いて記録した電位変化波形図である。

【図５】本発明の一体化複合電極の一実施例において、適当な電極を用いて長期に刺激電流を印加した後に、他の適当な電極を用いて記録した電位変化波形図である。

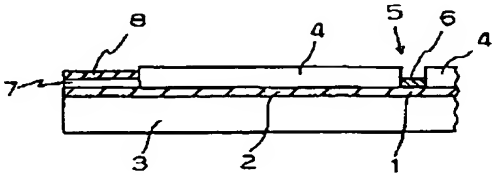
【図６】本発明の一体化複合電極の一実施例と電極表面を金でコートしていない点だけが異なる一体化複合電極を用いて、適当な電極を用いて長期に刺激電流を印加する前に、他の適当な電極を用いて記録した電位変化波形図である。

【図７】本発明の一体化複合電極の一実施例と電極表面を金でコートしていない点だけが異なる一体化複合電極を用いて、適当な電極を用いて長期に刺激電流を印加する前に、他の適当な電極を用いて記録した電位変化波形図である。

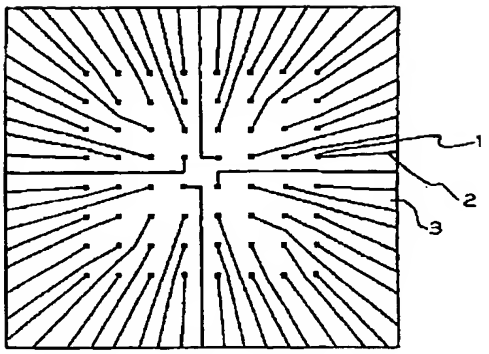
【符号の説明】

1　電極
2　リード線
3　絶縁基盤
4　絶縁層
5　孔
6　金
7　金
8　ニッケル

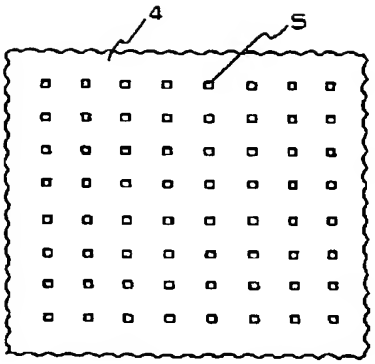
【図３】



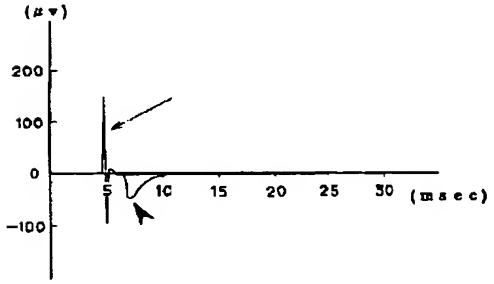
【図1】



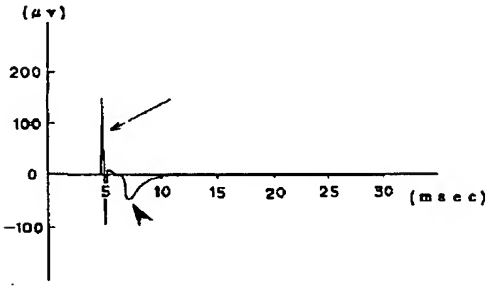
【図2】



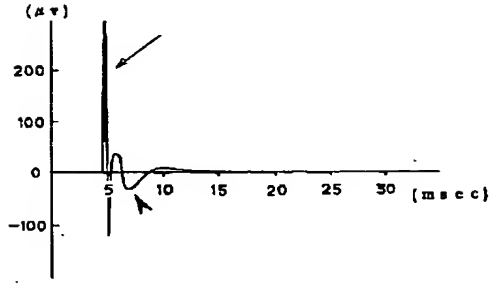
【図4】



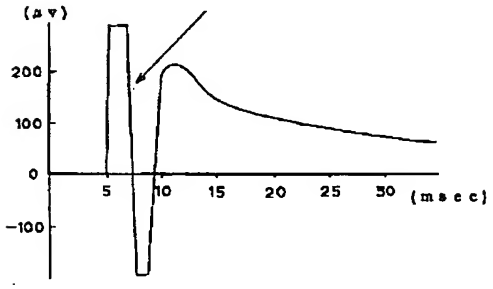
【図5】



【図6】



【図7】



フロントページの続き

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// H01B 5/14	Z			
		7638−4C	A61B 5/04 300 J	

* NOTICES *

1. This document has been translated by computer.So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] On an insulating base, the inter-electrode distance of the maximum contiguity carries out equality, and is, it has two or more electrodes, and the wiring section which arranged lead wire in the abbreviation radial from the aforementioned electrode, and the insulating layer which covers the aforementioned lead wire are prepared, and electrode area is 3x102. mum2 It is 4x104 above. mum2 It is the following ranges and the surface electrical resistance of the polar zone is the 10 ohm/cm 2. Unification composite electrode which is the following.
[Claim 2] The unification composite electrode according to claim 1 10-micrometer or more range of whose inter-electrode distance of the maximum contiguity is 1000 micrometers or less.
[Claim 3] The unification composite electrode according to claim 1 or 2 the insulating layer which covers lead wire has a hole on each electrode, and is [composite electrode] an insulating layer of the aforementioned insulating base mostly prepared in the whole surface [near the contact with the external circuit of lead wire].
[Claim 4] The unification composite electrode according to claim 1 to 3 to which two or more electrode cores are located in each intersection on the grid of 8x8.

[Translation done.]

* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] this invention relates to the unification composite electrode which is used in the field of the neurophysiology which measures electric measurement of living body activity, especially electric activity of a nerve cell and which has many electrodes.
[0002]
[Description of the Prior Art] In recent years, medical examination of a nerve cell, examination of the applicability as electric element, etc. have been performed actively. In case a nerve cell works, an action potential occurs. With change of the ionic permeability of a nerve cell, an action potential is produced, when the ion concentration of cell membrane inside and outside changes. And detection of nerve activity and examination are performed by measuring the potential change accompanying the ion concentration change near the nerve cell (namely, ion current) by the electrode.
[0003] In order to have measured electric activity of a nerve cell conventionally, usually the electric activity of a nerve cell at the time of inserting respectively in the inside of a cell or an intercellular the record electrode which consists of a glass electrode etc., and the stimulating electrode which consists of a metal electrode etc., and impressing stimulus current (or voltage) from a stimulating electrode was measured by the record electrode.
[0004] There are many strange methods, such as the so-called patch clump method for piercing a soma by the capillary-like glass suction electrode, flowing back the interior of a soma with the liquid in a glass suction electrode, giving an electrical signal from this glass suction electrode, and observing the electrical property of a cell membrane besides this.
[0005] Furthermore, this invention persons have proposed separately also about the method of impressing an electric stimulus to a cell and recording electric activity of a nerve cell, without stabbing an electrode into a cell by forming an electrode with a diameter of 15-20 micrometers by conductive matter, such as ITO (indium oxide tin), on an insulating base, and cultivating a nerve cell on this.
[0006] Moreover, the potential difference which generates the diameter of an electrode in inter-electrode when a constant-current stimulus is impressed to 20-200 micrometers, then a nerve cell became small as this improving method, as a result, destruction of ITO could not take place easily, and this invention persons have proposed the bird clapper separately that the observation over a long period of time is more possible.
[0007]
[Problem(s) to be Solved by the Invention] It was mainly spatial restrictions and the restrictions on operation precision, and since electrodes which cannot but become a remarkable size compared with a cell, such as a glass electrode, were used, two or more record electrodes inserted at once into one sample, and the multipoint simultaneous measurement which records electric activity of a nerve cell had the technical problem are very difficult, in the Prior art mentioned above and its strange method.
[0008] The degree of difficulty increased and the technical problem that observation between multicells could not be performed easily occurred as activity of many nerve cells needed to be

recorded simultaneously and point of measurement increased, in order to consider work of the whole neuron network.
[0009] Furthermore, since it was necessary to stab electrodes, such as glass and a metal, to the inside of a cell, or an intercellular, the technical problem that the injury done to a cell was large and measurement covering the long time of several hours or more could not be performed easily occurred.
[0010] On the other hand, if what formed the electrode [being circular (or square)] with a diameter (or one side) of 15-20 micrometers by conductive matter, such as ITO, on the insulating base is used, it will become observable [the signal transduction covering between multicells]. However, electrode area is 2 177 micrometers 2-400 micrometers. Since it was small, and the electrode resistance in a culture medium interface was set to several M omega and the stimulus was usually given by the constant current, when electrical stimulation was given over the long period of time on the big voltage which the very big potential difference will occur in inter-electrode, and will be built if electric resistance is large, destruction of ITO set, and there was a trouble that the observation over a long period of time was difficult for this reason.
[0011] Moreover, it is electrode area 300 micrometers 2-40000 micrometers 2 If it carries out, since the electrode resistance in a culture medium interface will become small, the potential difference generated in inter-electrode becomes a comparatively small thing. Even if it added stimulus current over the long period of time, destruction of ITO was not accepted in microscope. However, when stimulus current was impressed from a certain electrode and the potential change accompanying a stimulus was recorded by other electrodes, the big change to a record wave was seen before and after the long-term stimulus. That is, after the long-term stimulus, the influence (namely, artifact) stimulus current impression affects a record wave became larger than long-term stimulus before. It is thought that the cause of a changing wave shape is because an electrode front face polarizes. When the worst, electric activity of a nerve cell hid in the artifact, and measurement of it became impossible. Moreover, even when the artifact did not become so large, it is before and after a long-term stimulus, and there was a trouble that it became difficult to measure nerve activity intensity.
[0012] this invention solves this conventional trouble, performs multipoint simultaneous stimulus and measurement of a nerve cell etc. simple, makes possible the rear spring supporter of the signal transduction observation covering between multicells in several hours or more, and suppresses generating of the artifact accompanying stimulus current impression, and aims at offering the unification composite electrode which closes comparison of an electrography wave over long-term stimulus order, if possible.
[0013]
[Means for Solving the Problem] In order to solve the above-mentioned technical problem, the unification composite electrode of this invention The wiring section which the inter-electrode distance of the maximum contiguity carried out equality, was on the insulating base, was equipped with two or more electrodes, and arranged lead wire in the abbreviation radial from the aforementioned electrode, The insulating layer which covers the aforementioned lead wire is prepared, and electrode area is 3x102. mum2 It is 4x104 above. mum2 It is the following ranges and the surface electrical resistance of the polar zone is the 10 ohm/cm 2. It has the composition which is the following.
[0014] In the unification composite electrode of the aforementioned this invention, it is desirable that the inter-electrode distance of the maximum proximity is 10 micrometers or more 1000 micrometers or less. Moreover, in the unification composite electrode of the aforementioned this invention, the insulating layer which covers lead wire has a hole on each electrode, and it is desirable that it is the insulating layer of the aforementioned insulating base mostly prepared in the whole surface [near the contact with the external circuit of lead wire].
[0015] Moreover, in the unification composite electrode of the aforementioned this invention, it is desirable that two or more electrode cores are located in each intersection on the grid of 8x8.

[0016]
[Function] The wiring section which the inter-electrode distance of the maximum contiguity

carried out equality of the unification composite electrode of this invention, was on the insulating base, was equipped with two or more electrodes, and arranged lead wire in the abbreviation radial from the aforementioned electrode, The insulating layer which covers the aforementioned lead wire is prepared, and electrode area is 3x102. mum2 It is 4x104 above. mum2 It is the following ranges and the surface electrical resistance of the polar zone is the 10 ohm/cm 2. Since it is the following In case a signal is given to the nerve cell cultivated on the unification composite electrode of this invention and transfer of the signal of an intercellular is measured simultaneously By adjusting the inter-electrode distance of the maximum contiguity almost equally to the length of the nerve cell (namely, a soma, a dendrite, and a neurite) of the measuring object, and moreover making this electrode located in a line at equal intervals One soma arranges on an electrode and the probability that the soma through the cell salient to which it was extended from this soma is located on a ****** electrode becomes high. Therefore, transfer of the signal between ****** somata is detectable.

[0017] And since the lead wire lengthened from the electrode has been arranged to the abbreviation radial, the capacity component between lead wire (capacitance) decreases, collapse of the pulse signal wave which is an electrical signal can be made small compared with the case where lead wire has been arranged in parallel, for example and the time constant of a circuit becomes small, the responsibility to an early pulse signal improves and the flattery nature to the early component of nerve cell activity improves.

[0018] Furthermore, it is electrode area 3x102 mum2 It is 4x104 above. mum2 By adjusting in the following ranges, electrical stimulation can be given to a cell over the long time of several hours or more, and electric activity of a cell can be measured.

[0019] Moreover, the surface electrical resistance of the polar zone is the 10 ohm/cm 2. Since polarization on the front face of a stimulating electrode cannot happen easily in case it impresses stimulus current to a nerve cell by a certain electrode at a long period of time and the electric activity (potential change) of a nerve cell according to stimulus current is recorded by other electrodes, since it is the following, the influence (namely, artifact) stimulus current affects an electrography wave becomes small. After impressing stimulus current to a long period of time especially, the artifact is small, and since there is no change of a gestalt, electric activity of the nerve cell in long-term stimulus order can be compared.

[0020] Moreover, in the unification composite electrode of the aforementioned this invention, since the length of the neural spine of a nerve cell is generally this within the limits when the inter-electrode distance of the maximum contiguity considers as desirable 10-micrometer or more mode which is 1000 micrometers or less, possibility of a soma being located on an electrode and joining together through a neural spine is high, and serves as a convenient inter-electrode distance at measurement of a nerve cell.

[0021] In the unification composite electrode of the aforementioned this invention, moreover, the insulating layer which covers lead wire By having a hole on each electrode and considering as the desirable mode which is the insulating layer of the aforementioned insulating-layer base mostly prepared in the whole surface [near the contact surface with the external circuit of lead wire] An insulating material which consists of a photopolymer is used compared with the case where an insulating layer is alternatively prepared only on lead wire, and this resin is mostly applied to the whole surface. by the photo etching technique Since a required insulating layer can be easily formed by photo etching, such as opening a hole, so that the insulating layer on each electrode may be removed and an electrode may be exposed, production can be made easy and probability that an insulation is poor can be made small, it is desirable.

[0022] In the unification composite electrode of the aforementioned this invention, by being located in each intersection on the grid of 8x8, since two or more electrode cores can consider as the highest number of electrodes which can arrange lead wire in an abbreviation radial from the electrode of the aforementioned this invention, they are desirable further again.

[0023]

[Example] As an insulating base material with which this invention is presented, since it is necessary to carry out after [a cell culture] microscope observation, a transparent base is desirable, and although the organic substance which has transparency, such as mineral matters,

such as glass, such as quartz glass, lead glass, and boro-silicated glass, or a quartz, a polymethyl methacrylate or its copolymer, polystyrene, a polyvinyl chloride, polyester, polypropylene, a urea-resin, and melamine resin, is mentioned, when a mechanical strength and transparency are considered, a mineral matter is desirable.
[0024] As an electrode material with which this invention is presented, indium oxide tin (ITO), a tin oxide, Cr, Au, Cu, nickel, aluminum, etc. are usable, for example. If ITO or a tin oxide is used especially, although an electrode becomes the transparent thing which wore yellow slightly, and the visibility under the microscope of a nerve cell is good and advantageous on experiment operation, since ITO is especially right conductivity, it is desirable.
[0025] The same material also as the charge of a lead wire rod can be applied, and ITO is desirable too at the same reason as an electrode material. Although it does not limit especially, the thickness of such electrodes and lead wire is about about 500-5000A, usually, on an insulating base, carries out the vacuum evaporationo of such material, and can form them by etching at a desired pattern using a photoresist.
[0026] Moreover, as an insulating-layer material for insulating the lead wire with which this invention is presented, transparent resins, such as a polyimide (PI) resin, an epoxy resin, an acrylate resin, polyester resin, or polyamide resin, are mentioned, for example.
[0027] These resins are applied by the usual technique on lead wire, and an insulating layer is constituted. In addition, since pattern formation, such as opening a hole in a part for the insulating layer on an electrode in order to expose an electrode, as it mentioned above that insulating-layer material was photopolymers, such as photopolymerization nature, becomes possible, it is desirable.
[0028] It is desirable in order to show good growth especially, when insulating-layer material is PI and the cell to cultivate is a nerve cell. Also in PI, since a negative photograph sensitive polyimide (NPI) can form a hole in the shape of an electrode like the pattern formation of the wiring section using a photo etching process after applying a negative photograph sensitive polyimide all over abbreviation, it is still more desirable.
[0029] Moreover, although it does not limit especially, 0.1-10 micrometers is usually desirable, and 1-5 micrometers is [that the thickness of an insulating layer should just be the grade which can give insulation] still more desirable.
[0030] Using the unification composite electrode of this invention, the direct cell was cultivated and measurement record of the electric activity of a cell was carried out. Although the length of cell salients, such as the size or dendrite of a soma, and an axon, changes with kinds of a culture condition or cell, the inter-electrode distance of the maximum contiguity of a unification composite electrode has desirable 10-1000 micrometers. Since it approaches mutually too much that inter-electrode distance is less than 10 micrometers, the soma of adjacency **** probability decreases through a cell salient, and wiring of lead wire also becomes difficult. Moreover, if 1000 micrometers is exceeded, as for about 1000 micrometers of cell salients being extended by the plain-gauze cone, the probability of wiring of lead wire that eye a rare hatchet and a soma are located on an electrode will decrease. Since the length of a cell salient of the cell cultivated also on general conditions is about an average of 200-300 micrometers in the case of the central-nerves cell of mammalian, inter-electrode distance has desirable about 200-300 micrometers.
[0031] About electrode area, since it is necessary to make small resistance by the interface with culture medium in order to avoid the electrode destruction at the time of impressing electrical stimulation to a cell over a long period of time, the above size is required to some extent. However, if electrode area becomes large and resistance by the interface with culture medium becomes small, electric activity of the cell measured will become small and a S/N ratio will fall. That is, since it is I=V/R when [current value I] fixed, change of the potential V which will be measured if resistance R becomes small also becomes small. That is, electric activity of the cell measured becomes small and a S/N ratio falls. For this reason, it needs to be adjusted carefully, when it is the electrode of a circle configuration, a diameter is larger than 20 micrometers and 100 micrometers - 200 micrometers are especially desirable [electrode area] 200 micrometers or less.

[0032] Moreover, they are the 10 ohm/cm 2 about the surface electrical resistance of electrode section. In order to make it below, the coat of the metal was carried out to the ITO upper surface. As a coat material, although Ag, aluminum, Bi, Au, Cu, Cr, Pt, Co, etc. are usable, if the toxic lowness to a nerve cell is taken into consideration, use of Au and Pt is desirable. Although not limited, especially the thickness of a coat is about about 500A, usually, on an insulating base, carries out the vacuum evaporationo of such material, and can form them by etching at a desired pattern using a photoresist.
[0033] Furthermore, according to the desirable mode which this invention mentioned above, in order to detect electric activity from a ****** soma at the same time it gives electrical stimulation to the soma cultivated on the unification composite electrode, the hole in the insulating layer of a unification composite electrode is formed in order to expose an electrode, and is located in an electrode core.
[0034] Moreover, by arranging in an abbreviation radial the lead wire lengthened from the electrode, the capacity component between lead wire is lost, a noise decreases, and the accuracy of measurement improves. Moreover, it is desirable to prepare an electrode in each intersection on the grid of 8x8 from a viewpoint of the electrode core of the unification composite electrode of this invention being able to wire a radial in lead wire as it is the composition of being located in each intersection on the shape of a concentric circle and 8x8 or less grid, and it constituting as many electrodes as possible especially, and performing multipoint simultaneous stimulus and record.
[0035] Below, a concrete example explains the unification composite electrode of this invention to a detail further.
Example 1 drawing 1 is the plan having shown the pattern of the wiring section in the state where there is no insulating layer of an electrode 1 and the unification composite electrode of this example in which lead wire 2 was formed on the insulating base 3. some plans of only the insulating layer formed on the member which showed drawing 2 by drawing 1 -- it is a notching view Drawing 3 is some cross sections of the unification composite electrode of this example. It explains referring to these drawings below.
[0036] First, production of the composite-electrode wiring section is described. As a strong transparent insulating material of a mechanical strength, the insulating base 3 of a unification composite electrode used 50x50x1mm hard glass (it is the same below "IWAKI CODE 7740 GLASS" [the Iwaki Glass Co., Ltd. make]).
[0037] ITO was used for the material of an electrode 1 and lead wire 2, vacuum evaporationo was carried out to about 1000A ** the whole surface on the insulating base 3 of the aforementioned hard glass, and it washed after that. Next, the core of each electrode 1 is located in each intersection on the grid of 8x8 (position as shown by 5 of drawing 2). So that it may become the electrode 1 of the configuration where the pitch of the electrode of the maximum contiguity of each electrode was equal, and lead wire 2 was moreover extended to the radial, and the pattern of lead wire 2 It exposed using the photoresist, and the photoresist was removed after **********ing ITO in the solution mixed by the volume ratio of pure water 50, a hydrochloric acid 50, and a nitric acid 1. In the width of face of 60 micrometers and lead wire 2, 30 micrometers and the electrode pitch formed [the diameter of an electrode 1] the 300-micrometer wiring section.
[0038] Subsequently, as an insulating layer 4, the spin coat of the negative photograph sensitive polyimide (it omits Following NPI) was carried out so that the thickness after dryness might be set to 1 micrometer, and exposure formation of the insulating-layer pattern was carried out so that the with an one-side square [50-micrometer square] hole 5 might be made at the center of each electrode of the wiring section, as shown in drawing 2 . Furthermore, gold 6 was deposited so that it might become a part for the outcrop of each electrode (namely, with an one-side square [50-micrometer square] interior) with 500A of thickness.
[0039] The coat of the contact of the electrode 1 of lead wire 2 and the external circuit of the portion near the edge of opposite direction was carried out with gold 7 and nickel 8, and it raised endurance. In addition, although ITO was used for the electrode 1 and the portion of lead 2 and gold was used for the insulating layer in this example at NPI and electrode surface coat material,

it was already said that the material to be used is not limited to these.
[0040] Moreover, the process for constituting the unification composite electrode of this invention is not limited to the method of this example.
Cultivation of the nerve cell on an example 2, next a unification composite electrode is described.
[0041] It carried out like an example 1 and rat cerebrum visual cortex was cultivated as a nerve cell on the constituted unification composite electrode. Hereafter, cultivation is described in detail.
(b) Extract the brain of the embryo of SD rat which passed 16 - 18 days after pregnancy, and dip in the ice-cooled Hanks balance **** (it omits Following HBBS).
(b) Start visual cortex from the brain under ice-cooling HBBS, and move into eagle MEM (it omits Following MEM) liquid.
(c) In MEM liquid, it is fine as much as possible, and cut visual cortex so that it may become 0.2mm angle at the maximum.
(d) Put the visual cortex cut finely into a centrifugation tube (test tube for centrifugal separation), and distribute in this liquid of optimum dose after washing 3 times by HBBS (it abbreviates to CMF-HBBS below) which does not contain calcium and magnesium.
(e) Add the CMF-HBBS solution (0.25 % of the weight) of a trypsin into the centrifugation tube of the above-mentioned (**), and double the whole quantity. while agitating gently -- 37 degrees C -- for 15 minutes to 20 minutes -- constant temperature -- it maintained at the state and the enzyme reaction was made to perform
(**) Double [be / under / centrifugation tube / which passed through the above-mentioned (e) the DMEM/F-12 mixture culture medium which mixed the Dulbecco change Eagle's medium (DMEM) and HamF-12 culture medium containing fetal-calf-serum (FCS) 10vol.% by the volume ratio of 1 to 1] adding / it] the whole quantity further. With Pasteur pipette which roasted the nose of cam over the burner and made aperture small, pipetting is repeated gently (about a maximum of 20 times), and a cell is unfolded.
(**) -- at-long-intervals heart separation is performed by 9806.65 m/sec2 (namely, 1000g) for about 5 minutes A supernatant liquid is thrown away after a centrifugal separation end, and sedimentation is suspended in the DMEM/F-12 mixture culture medium containing FCS5vol.%.
(h) Repeat the above-mentioned (**) and (**) twice [more] (a total of 3 times).
(i) Suspend the sedimentation finally obtained in the DMEM/F-12 mixture culture medium containing 5vol.%FCS, and measure the cell concentration in suspension using the number board of erythrocytometer. The same culture medium is used and it is cell concentration Two to 4x106 It adjusts so that it may be set to an individual/ml.
(j) Add 500micro of DMEM/F-12 mixture culture media I which contain 5vol(s).%FCS beforehand in the well for cell cultures which constituted the cylinder made of a plastic with a diameter, and a height of 6mm by doubling the center of a composite electrode, and the center of a plastic cylinder, and pasting up on the unification composite electrode, and it is CO2. It warms within the incubator (air concentration 95vol.%, CO2 concentration 5vol.%, relative humidity of 97%, temperature of 37
(**) Add calmly 100micro of suspension I which adjusted cell concentration into the well of the above-mentioned (j), and it is CO2 again. It puts into an incubator.
The moiety of a culture medium is exchanged for a new thing three days after from operation of the (**) above-mentioned (**). An exchange culture medium uses the DMEM/F-12 mixture culture medium which does not contain FCS.
(**) Perform the same culture-medium exchange as the above day by day [4 - 5] henceforth.
[0042] By operation of these series, the nerve cell of rat cerebrum visual cortex was able to be cultivated on the unification composite electrode. The cell was grown good also on the electrode which deposited an insulating-layer (NPI) top or platinum black. Therefore, when using the electrode in a suitable position as a stimulating electrode or a record electrode, simultaneous multipoint measurement of nerve cell electrical-and-electric-equipment activity was possible.
[0043] Moreover, the example which recorded the electric response (potential change) of a nerve cell by the electrode which is in a suitable position before and after giving a constant-

current stimulus of 100microA over one week through the electrode in the suitable position of the unification composite electrode of one example of this invention by the frequency of 1Hz is shown in drawing 4 and drawing 5 . Drawing 4 shows record of an electric response of the nerve cell before a stimulus, and drawing 5 shows record of an electric response of the nerve cell after a stimulus.

[0044] Furthermore, the example which recorded the electric response of a nerve cell before and after adding a long-term stimulus to drawing 6 and drawing 7 on the same conditions as the above using the unification composite electrode which has not carried out the coat of the electrode front face withgold is shown. Drawing 6 shows record of an electric response of the nerve cell before a stimulus, and drawing 7 shows record of an electric response of the nerve cell after a stimulus.

[0045] The artifact which generated the arrow with stimulus current impression in drawing 7 from drawing 4 , and Yato show potential change generated by electric activity of a nerve cell. By the case where the unification composite electrode of one example of this invention of drawing 4 is used, generating of the artifact is suppressed to the thing with generating of the artifact large when the unification composite electrode which has not carried out the coat of the electrode front face withgold is used so that drawing 6 may show.

[0046] Moreover, when the unification composite electrode which has not carried out the coat of the electrode front face withgold was used so that drawing 7 may show, generating of the artifact was larger than stimulus before, electric activity of a nerve cell hid in the artifact, and measurement of it became impossible. To it, like the case where it is shown by drawing 4 , generating of the artifact is suppressed and electric activity of a nerve cell was fully able to be recorded by the case where the unification composite electrode of one example of this invention of drawing 5 is used.

[0047] In addition, the cultivation of a nerve cell has many strange methods besides this example, and is not limited to this example.

[0048]

[Effect of the Invention] The unification composite electrode of this invention can offer the unification composite electrode which cultivation of a nerve cell was possible, and could realize simultaneous multipoint measurement of the nerve cell electrical-and-electric-equipment activity which was conventionally impossible or very difficult, and long-term observation of the signal transduction covering a multicell of several hours or more, and was excellent in responsibility as explained above.

[0049] Moreover, when 10-micrometer or more range of the inter-electrode distance of the maximum contiguity is 1000 micrometers or less, possibility of each soma being located on each electrode, and joining together through a neural spine is made highly, and can consider as a convenient unification composite electrode at measurement of a nerve cell.

[0050] Moreover, the insulating material which consists of a photopolymer by the insulating layer which covers lead wire having a hole on each electrode, and being the insulating layer of the aforementioned insulation base mostly prepared in the whole surface [near the contact with the external circuit of lead wire] is used, this resin is mostly applied to the whole surface, a required insulating-layer pattern can be easily formed by the photo etching technique, production is easy, and it can consider as a unification composite electrode small [of the probability that an insulation is poor].

[0051] Moreover, the surface electrical resistance of each electrode section is low, and since the coat is carried out by the low matter of cytotoxicity, after adding stimulus current using a suitable electrode and adding a stimulus to a long period of time over recording potential change using other suitable electrodes, there is little polarization of an electrode and it can consider as the unification composite electrode in which the stable record is possible.

[0052] Moreover, two or more electrode cores can consider as the highest number of electrodes which can arrange lead wire in an abbreviation radial from the electrode of the aforementioned this invention by being located in each intersection on the grid of 8x8.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the plan having shown the pattern in the state where there is no insulating layer of an electrode and the unification composite electrode of this invention in which lead wire was formed on the insulating base of one example of this invention.
[Drawing 2] some plans of only the insulating layer of one example of the unification composite electrode of this invention -- it is a notching view
[Drawing 3] They are some cross sections of one example of the unification composite electrode of this invention.
[Drawing 4] In one example of the unification composite electrode of this invention, before impressing stimulus current to a long period of time using a suitable electrode, it is the potential change wave form chart which used and recorded other suitable electrodes.
[Drawing 5] In one example of the unification composite electrode of this invention, after impressing stimulus current to a long period of time using a suitable electrode, it is the potential change wave form chart which used and recorded other suitable electrodes.
[Drawing 6] Before impressing stimulus current to a long period of time using a suitable electrode using the unification composite electrode from which only the point which has not carried out the coat of one example and electrode front face of a unification composite electrode of this invention withgold differs, it is the potential change wave form chart which used and recorded other suitable electrodes.
[Drawing 7] Before impressing stimulus current to a long period of time using a suitable electrode using the unification composite electrode from which only the point which has not carried out the coat of one example and electrode front face of a unification composite electrode of this invention withgold differs, it is the potential change wave form chart which used and recorded other suitable electrodes.
[Description of Notations]
1 Electrode
2 Lead Wire
3 Insulating Base
4 Insulating Layer
5 Hole
6 Gold
7 Gold
8 Nickel

[Translation done.]